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Dahlquist, Anders Stahl, Ulf Lenman, Marit Banas, Antoni Ronne, Hans

<120> A new class of enzymes in the biosynthetic pathway for the production of triacylglycerol and recombinant DNA molecules encoding these enzymes

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<141> 2000-03-30

<150> EP 99106656.4 <151> 1999-04-01

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Gln Tyr Val Thr Glu Arg Ile Thr Gly Pro Leu Pro Asp Pro Pro Gly

90 95 85 Val Lys Leu Lys Lys Glu Gly Leu Lys Ala Lys His Pro Val Val Phe 105 Ile Pro Gly Ile Val Thr Gly Gly Leu Glu Leu Trp Glu Gly Lys Gln 120 Cys Ala Asp Gly Leu Phe Arg Lys Arg Leu Trp Gly Gly Thr Phe Gly Glu Val Tyr Lys Arg Pro Leu Cys Trp Val Glu His Met Ser Leu Asp Asn Glu Thr Gly Leu Asp Pro Ala Gly Ile Arg Val Arg Ala Val Ser Gly Leu Val Ala Ala Asp Tyr Phe Ala Pro Gly Tyr Phe Val Trp Ala Val Leu Ile Ala Asn Leu Ala His Ile Gly Tyr Glu Glu Lys Asn Met Tyr Met Ala Ala Tyr Asp Trp Arg Leu Ser Phe Gln Asn Thr Glu Val Arg Asp Gln Thr Leu Ser Arg Met Lys Ser Asn Ile Glu Leu Met Val Ser Thr Asn Gly Gly Lys Lys Ala Val Ile Val Pro His Ser Met Gly Val Leu Tyr Phe Leu His Phe Met Lys Trp Val Glu Ala Pro Ala Pro Leu Gly Gly Gly Gly Pro Asp Trp Cys Ala Lys Tyr Ile Lys Ala Val Met Asn Ile Gly Gly Pro Phe Leu Gly Val Pro Lys Ala Val Ala Gly Leu Phe Ser Ala Glu Ala Lys Asp Val Ala Val Ala Arg Ala Ile 310 Ala Pro Gly Phe Leu Asp Thr Asp Ile Phe Arg Leu Gln Thr Leu Gln 330 325 His Val Met Arg Met Thr Arg Thr Trp Asp Ser Thr Met Ser Met Leu 345 Pro Lys Gly Gly Asp Thr Ile Trp Gly Gly Leu Asp Trp Ser Pro Glu Lys Gly His Thr Cys Cys Gly Lys Lys Gln Lys Asn Asn Glu Thr Cys Gly Glu Ala Gly Glu Asn Gly Val Ser Lys Lys Ser Pro Val Asn Tyr 390

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Pro Asn His Thr Cys Arg Asp Val Trp Thr Glu Tyr His Asp Met Gly 435 440 445

Ile Ala Gly Ile Lys Ala Ile Ala Glu Tyr Lys Val Tyr Thr Ala Gly 450 455 460

Glu Ala Ile Asp Leu Leu His Tyr Val Ala Pro Lys Met Met Ala Arg 465 470 475 480

Gly Ala Ala His Phe Ser Tyr Gly Ile Ala Asp Asp Leu Asp Asp Thr 485 490 495

Lys Tyr Gln Asp Pro Lys Tyr Trp Ser Asn Pro Leu Glu Thr Lys Leu 500 505 510

Pro Asn Ala Pro Glu Met Glu Ile Tyr Ser Leu Tyr Gly Val Gly Ile 515 520 525

Pro Thr Glu Arg Ala Tyr Val Tyr Lys Leu Asn Gln Ser Pro Asp Ser 530 535 540

Cys Ile Pro Phe Gln Ile Phe Thr Ser Ala His Glu Glu Asp Glu Asp 545 555 560

Ser Cys Leu Lys Ala Gly Val Tyr Asn Val Asp Gly Asp Glu Thr Val 565 570 575

Pro Val Leu Ser Ala Gly Tyr Met Cys Ala Lys Ala Trp Arg Gly Lys 580 585 590

Thr Arg Phe Asn Pro Ser Gly Ile Lys Thr Tyr Ile Arg Glu Tyr Asn 595 600 605

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Arg Lys Ser Lys Phe Gly Lys Arg Leu Asn Phe Ile Leu Gly Ala Ile
50 55 60

Leu Gly Ile Cys Gly Ala Phe Phe Phe Ala Val Gly Asp Asp Asn Ala 65 70 75 80

Val Phe Asp Pro Ala Thr Leu Asp Lys Phe Gly Asn Met Leu Gly Ser 85 90 95

Ser Asp Leu Phe Asp Asp Ile Lys Gly Tyr Leu Ser Tyr Asn Val Phe
100 105 110

Lys Asp Ala Pro Phe Thr Thr Asp Lys Pro Ser Gln Ser Pro Ser Gly
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Asn Glu Val Gln Val Gly Leu Asp Met Tyr Asn Glu Gly Tyr Arg Ser 130 135 140

Asp His Pro Val Ile Met Val Pro Gly Val Ile Ser Ser Gly Leu Glu 145 150 155 160

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Ile Thr Gly Tyr Trp Ile Trp Ser Lys Val Ile Glu Asn Leu Ala Ala 225 230 235 240

Ile Gly Tyr Glu Pro Asn Asn Met Leu Ser Ala Ser Tyr Asp Trp Arg 245 250 255

Leu Ser Tyr Ala Asn Leu Glu Glu Arg Asp Lys Tyr Phe Ser Lys Leu

260 265 270 Lys Met Phe Ile Glu Tyr Ser Asn Ile Val His Lys Lys Lys Val Val Leu Ile Ser His Ser Met Gly Ser Gln Val Thr Tyr Tyr Phe Phe Lys Trp Val Glu Ala Glu Gly Tyr Gly Asn Gly Gly Pro Thr Trp Val Asn 315 Asp His Ile Glu Ala Phe Ile Asn Ile Ser Gly Ser Leu Ile Gly Ala Pro Lys Thr Val Ala Ala Leu Leu Ser Gly Glu Met Lys Asp Thr Gly 340 Ile Val Ile Thr Leu Asn Ile Leu Glu Lys Phe Phe Ser Arg Ser Glu Arg Ala Met Met Val Arg Thr Met Gly Gly Val Ser Ser Met Leu Pro Lys Gly Gly Asp Val Ala Pro Asp Asp Leu Asn Gln Thr Asn Phe Ser Asn Gly Ala Ile Ile Arg Tyr Arg Glu Asp Ile Asp Lys Asp His Asp Glu Phe Asp Ile Asp Asp Ala Leu Gln Phe Leu Lys Asn Val Thr Asp 425 Asp Asp Phe Lys Val Met Leu Ala Lys Asn Tyr Ser His Gly Leu Ala Trp Thr Glu Lys Glu Val Leu Lys Asn Asn Glu Met Pro Ser Lys Trp Ile Asn Pro Leu Glu Thr Ser Leu Pro Tyr Ala Pro Asp Met Lys Ile Tyr Cys Val His Gly Val Gly Lys Pro Thr Glu Arg Gly Tyr Tyr Tyr Thr Asn Asn Pro Glu Gly Gln Pro Val Ile Asp Ser Ser Val Asn Asp Gly Thr Lys Val Glu Asn Gly Ile Val Met Asp Asp Gly Asp Gly Thr Leu Pro Ile Leu Ala Leu Gly Leu Val Cys Asn Lys Val Trp Gln Thr Lys Arg Phe Asn Pro Ala Asn Thr Ser Ile Thr Asn Tyr Glu Ile Lys His Glu Pro Ala Ala Phe Asp Leu Arg Gly Gly Pro Arg Ser Ala Glu 570

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Leu Asp Arg Glu Tyr Lys Pro Ser Ser Val Trp Cys Ser Ser Trp Leu
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Lys Ala Leu Glu Lys Lys Cys Gly Tyr Val Asn Asp Gln Thr Ile Leu 145 150 155 160

Gly Ala Pro Tyr Asp Phe Arg Tyr Gly Leu Ala Ala Ser Gly His Pro 165 170 175

Ser Arg Val Ala Ser Gln Phe Leu Gln Asp Leu Lys Gln Leu Val Glu 180 185 190

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Ser Trp Arg Arg Lys Tyr Ile Lys His Phe Val Ala Leu Ala Ala Pro

19

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Phe Ala Ser Thr Gln Leu Arg Ala Trp Ser Ile Leu Asp Cys Pro Tyr 50 55 60

• • •

Thr Pro Leu Asp Phe Asn Pro Leu Asp Leu Val Trp Leu Asp Thr Thr Lys Leu Leu Ser Ala Val Asn Cys Trp Phe Lys Cys Met Val Leu Asp Pro Tyr Asn Gln Thr Asp His Pro Glu Cys Lys Ser Arg Pro Asp Ser Gly Leu Ser Ala Ile Thr Glu Leu Asp Pro Gly Tyr Ile Thr Gly Pro 120 Leu Ser Thr Val Trp Lys Glu Trp Leu Lys Trp Cys Val Glu Phe Gly Ile Glu Ala Asn Ala Ile Val Ala Val Pro Tyr Asp Trp Arg Leu Ser 150 Pro Thr Lys Leu Glu Glu Arg Asp Leu Tyr Phe His Lys Leu Lys Leu Thr Phe Glu Thr Ala Leu Lys Leu Arg Gly Gly Pro Ser Ile Val Phe Ala His Ser Met Gly Asn Asn Val Phe Arg Tyr Phe Leu Glu Trp Leu Arg Leu Glu Ile Ala Pro Lys His Tyr Leu Lys Trp Leu Asp Gln His Ile His Ala Tyr Phe Ala Val Gly Ala Pro Leu Leu Gly Ser Val Glu Ala Ile Lys Ser Thr Leu Ser Gly Val Thr Phe Gly Leu Pro Val Ser Glu Gly Thr Ala Arg Leu Leu Ser Asn Ser Phe Ala Ser Ser Leu Trp Leu Met Pro Phe Ser Lys Asn Cys Lys Gly Asp Asn Thr Phe Trp Thr His Phe Ser Gly Gly Ala Ala Lys Lys Asp Lys Arg Val Tyr His Cys Asp Glu Glu Glu Tyr Gln Ser Lys Tyr Ser Gly Trp Pro Thr Asn Ile Ile Asn Ile Glu Ile Pro Ser Thr Ser Ala Arg Glu Leu Ala Asp Gly Thr Leu Phe Lys Ala Ile Glu Asp Tyr Asp Pro Asp Ser Lys Arg Met Leu His Gln Leu Lys Lys Tyr Val Pro Phe Phe Val Ile Arg Asn Ile 360 Ala His Arg Ser Ser Leu Ala Gly Phe Leu Leu Tyr His Asp Asp Pro 370

Val Phe Asn Pro Leu Thr Pro Trp Glu Arg Pro Pro Ile Lys Asn Val 385 390 395 400

Phe Cys Ile Tyr Gly Ala His Leu Lys Thr Glu Val Gly Tyr Tyr Phe 405 410 415

Ala Pro Ser Gly Lys Pro Tyr Pro Asp Asn Trp Ile Ile Thr Asp Ile 420 425 430

Ile Tyr Glu Thr Glu Gly Ser Leu Val Ser Arg Ser Gly Thr Val Val
435
440
445

Asp Gly Asn Ala Gly Pro Ile Thr Gly Asp Glu Thr Val Pro Tyr His 450 455 460

Ser Leu Ser Trp Cys Lys Asn Trp Leu Gly Pro Lys Val Asn Ile Thr 465 470 475 480

Met Ala Pro Gln Ile Leu Ile Gly Lys Ile Lys Gln Gln Pro Glu His
485 490 495

Asp Gly Ser Asp Val His Val Glu Leu Asn Val Asp His Glu His Gly
500 505 510

Ser Asp Ile Ile Ala Asn Met Thr Lys Ala Pro Arg Val Lys Tyr Ile 515 520 525

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35 40 45

Phe Arg Leu Trp Phe Asp Ala Ala Val Leu Leu Ser Pro Phe Thr Arg 50 55 60

Cys Phe Ser Asp Arg Met Met Leu Tyr Tyr Asp Pro Asp Leu Asp Asp 65 70 75 80

Tyr Gln Asn Ala Pro Gly Val Gln Thr Arg Val Pro His Phe Gly Ser 85 90 95

Thr Lys Ser Leu Leu Tyr Leu Asp Pro Arg Leu Arg Asp Ala Thr Ser

22

100 105 110 Tyr Met Glu His Leu Val Lys Ala Leu Glu Lys Lys Cys Gly Tyr Val Asn Asp Gln Thr Ile Leu Gly Ala Pro Tyr Asp Phe Arg Tyr Gly Leu 135 Ala Ala Ser Gly His Pro Ser Arg Val Ala Ser Gln Phe Leu Gln Asp Leu Lys Gln Leu Val Glu Lys Thr Ser Ser Glu Asn Glu Gly Lys Pro 170 Val Ile Leu Leu Ser His Ser Leu Gly Gly Leu Phe Val Leu His Phe Leu Asn Arg Thr Thr Pro Ser Trp Arg Arg Lys Tyr Ile Lys His Phe Val Ala Leu Ala Ala Pro Trp Gly Gly Thr Ile Ser Gln Met Lys Thr Phe Ala Ser Gly Asn Thr Leu Gly Val Pro Leu Val Asn Pro Leu Leu Val Arg Arg His Gln Arg Thr Ser Glu Ser Asn Gln Trp Leu Leu Pro Ser Thr Lys Val Phe His Asp Arg Thr Lys Pro Leu Val Val Thr Pro Gln Val Asn Tyr Thr Ala Tyr Glu Met Asp Arg Phe Phe Ala Asp Ile Gly Phe Ser Gln Gly Val Val Pro Tyr Lys Thr Arg Val Leu Pro Leu Thr Glu Glu Leu Met Thr Pro Gly Val Pro Val Thr Cys Ile Tyr Gly Arg Gly Val Asp Thr Pro Glu Val Leu Met Tyr Gly Lys Gly Phe Asp Lys Gln Pro Glu Ile Lys Tyr Gly Asp Gly Asp Gly Thr Val Asn Leu Ala Ser Leu Ala Ala Leu Lys Val Asp Ser Leu Asn Thr Val Glu Ile Asp Gly Val Ser His Thr Ser Ile Leu Lys Asp Glu Ile Ala Leu 375 Lys Glu Ile 385

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Val Glu His Met Ser Leu Asp Asn Glu Thr Gly Leu Asp Pro Ala Gly 50 60

Ile Arg Val Arg Ala Val Ser Gly Leu Val Ala Ala Asp Tyr Phe Ala 65 70 75 80

Pro Gly Tyr Phe Val Trp Ala Val Leu Ile Ala Asn Leu Ala His Ile 85 90 95

Gly Tyr Glu Glu Lys Asn Met Tyr Met Ala Ala Tyr Asp Trp Arg Leu 100 105 110

Ser Phe Gln Asn Thr Glu Arg Asp Gln Thr Leu Ser Arg Met Lys Ser 115 120 125

Asn Ile Glu Leu Met Val Ser Thr Asn Gly Gly Lys Lys Ala Val Ile 130 135 140

Val Pro His Ser Met Gly Val Leu Tyr Phe Leu His Phe Met Lys Trp 145 150 155 160

Val Glu Ala Pro Ala Pro Leu Gly Gly Gly Gly Pro Asp Trp Cys 165 170 175

Ala Lys Tyr Ile Lys Ala Val Met Asn Ile Gly Gly Pro Phe Leu Gly
180 185 190

Val Pro Lys Ala Val Ala Gly Leu Phe Ser Ala Glu Ala Lys Asp Met 195 200 205

Arg Met Thr Arg Thr Trp Asp Ser Thr Met Ser Met Leu Pro Lys Gly 210 215 220

Gly Asp Thr Ile Trp Gly Gly Leu Asp Trp Ser Pro Glu Leu Pro Asn 225 230 235 240

Ala Pro Glu Met Glu Ile Tyr Ser Leu Tyr Gly Val Gly Ile Pro Thr 245 250 255

Glu Arg Ala Tyr Val Tyr Lys Leu Asn Gln Ser Pro Asp Ser Cys Ile 260 265 270

Pro Phe Gln Ile Phe Thr Ser Ala His Glu Glu Asp Glu Asp Ser Cys 275 280 285 Leu Lys Ala Gly Val Tyr Asn Val Asp Gly Asp Glu Thr Val Pro Val 290 295 Leu Ser Ala Gly Tyr Met Cys Ala Lys Ala Trp Arg Gly Lys Thr Arg Phe Asn Pro Ser Gly Ile Lys Thr Tyr Ile Arg Glu Tyr Asn His Ser 325 Pro Pro Ala Asn Leu Leu Glu Gly Arg Gly Thr Gln Ser Gly Ala His Val Asp Ile Met Gly Asn Phe Ala Leu Ile Glu Asp Ile Met Arg Val Ala Ala Gly Gly Asn Gly Ser Asp Ile Gly His Asp Gln Val His Ser 375 Gly Ile Phe Glu Trp 385 <210> 18 <211> 402 <212> DNA <213> Arabidopsis thaliana <220> <221> CDS <222> (120)..(401) <221> Unsure <222> 1..401 <223> n is c, g, a, t or u. <221> Unsure <222> 1..401 <223> Xaa = unknown <400> 18 agaaacagct ctttqtctct ctcqactqat ctaacaatcc ctaatctqtq ttctaaattc 60 ctggacgaga tttgacaaag tccgtatagc ttaacctggt ttaatttcaa gtgacagat atg ccc ctt att cat cgg aaa aag ccg acg gag aaa cca tcg acg ccg 167 Met Pro Leu Ile His Arg Lys Lys Pro Thr Glu Lys Pro Ser Thr Pro cca tct gaa gag gtg gtg cac gat gag gat tcg caa aag aaa cca cac 215 Pro Ser Glu Glu Val Val His Asp Glu Asp Ser Gln Lys Lys Pro His 20 gaa tot too aaa too cac cat aag naa tog aac gga gga ggg aag tgg 263 Glu Ser Ser Lys Ser His His Lys Xaa Ser Asn Gly Gly Gly Lys Trp 35 teg tgc atc gat tet tgt tgt tgg ttc att ggg tgt gtg tgt gta acc Ser Cys Ile Asp Ser Cys Cys Trp Phe Ile Gly Cys Val Cys Val Thr

24

41 1 7

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